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*DB=USPT,DWPI; PLUR=YES; OP=ADJ*

L3 ('t-cell' or 't-lymphocyte\$)same (deplet\$ or mask\$) same (antibod\$)same  
(hour\$)

20 L3*DB=DWPI; PLUR=YES; OP=ADJ*

L2 (marrow)same (deplet\$ or mask\$) same (antibod\$)same (hour\$)

1 L2*DB=USPT; PLUR=YES; OP=ADJ*

L1 (marrow)same (deplet\$ or mask\$) same (antibod\$)same (hour\$)

30 L1

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L1: Entry 25 of 30

File: USPT

Apr 7, 1998

DOCUMENT-IDENTIFIER: US 5736137 A

TITLE: Therapeutic application of chimeric and radiolabeled antibodies to human B lymphocyte restricted differentiation antigen for treatment of B cell lymphoma

Detailed Description Text (11):

Introduction of the immunologically active chimeric anti-CD20 antibodies in these dose ranges can be carried out as a single treatment or over a series of treatments. With respect to chimeric antibodies, it is preferred that such introduction be carried out over a series of treatments; this preferred approach is predicated upon the treatment methodology associated with this disease. While not wishing to be bound by any particular theory, because the immunologically active chimeric anti-CD20 antibodies are both immunologically active and bind to CD20, upon initial introduction of the immunologically active chimeric anti-CD20 antibodies to the individual, peripheral blood B cell depletion will begin; we have observed a nearly complete depletion within about 24 hours post treatment infusion. Because of this, subsequent introduction(s) of the immunologically active chimeric anti-CD20 antibodies (or radiolabeled anti-CD20 antibodies) to the patient is presumed to: a) clear remaining peripheral blood B cells; b) begin B cell depletion from lymph nodes; c) begin B cell depletion from other tissue sources, eg, bone marrow, tumor, etc. Stated again, by using repeated introductions of the immunologically active chimeric anti-CD20 antibodies, a series of events take place, each event being viewed by us as important to effective treatment of the disease. The first "event" then, can be viewed as principally directed to substantially depleting the patient's peripheral blood B cells; the subsequent "events" can be viewed as either principally directed to simultaneously or serially clearing remaining B cells from the system clearing lymph node B cells, or clearing other tissue B cells.

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L3: Entry 2 of 20

File: USPT

Mar 27, 2001

DOCUMENT-IDENTIFIER: US 6207156 B1

TITLE: Specific antibodies and antibody fragments

Brief Summary Text (51):

An example of a T-cell proliferation assay is disclosed in Walunas et al., J. Exp. Med V. 183, p. 2541-2550 (1996). Briefly, lymph node cells are isolated and enriched for T-cells by passage over a nylon wool column and T-cell purity is evaluated by flow cytometry using an anti-CD3 monoclonal antibody. The T-cells are plated at a density of 2.times.10.sup.5 /well in the presence of 1.times.10.sup.5 irradiated syngeneic, erythrocyte-depleted B6 splenocytes. Once the cells are prepared, two assay conditions are set up. A first assay condition involves incubating the T-cell mixture with 0.1 mg/ml of anti-CD3 and 1.0 mg/ml of anti-CD28 for 72 hours at 37. The second assay condition involves incubating the T-cell mixture with 0.1 mg/ml of anti-CD3 alone for 72 hours at 37. Each of the assay conditions are mixed with either control Ig (50 mg/ml), anti-CTLA-4 (50 mg/ml) (such as the deposited monoclonal antibody), or the test peptide of the invention (50 mg/ml). During the last 16 hours of the incubation the mixture is pulsed with 1 mCi/well [.sup.3 H] thymidine. The samples are then counted on a scintillation counter and the proliferation of the cells is measured as a function of the [.sup.3 H] thymidine incorporated into the cells.

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L3: Entry 5 of 20

File: USPT

Jan 11, 2000

DOCUMENT-IDENTIFIER: US 6013519 A

TITLE: Monoclonal antibodies to antigens expressed by hematopoietic facilitatory cells

Detailed Description Text (28):

In order to screen and select for MAb directed to FC, a preparation of donor bone marrow cells was reacted with hybridoma supernatants prior to their injection into allogeneic mouse recipients. Mixed allogeneic chimerism in the recipients was used as an indicator of the presence of MAb capable of depleting FC function. To prepare mixed chimeras, bone marrow from the long bones of syngeneic (B10) mice and allogeneic (B10.BR) mice were harvested. The mice were euthanized with CO<sub>2</sub> narcosis, prepared with 70% alcohol, and the long hind bone (femora and tibia) removed. The marrow was flushed from the bones using medium 199 (Gibco Laboratories Life Technology, Inc., Grand Island, N.Y.) supplemented with 50  $\mu$ g/ml of gentamicin using a 22-gauge needle. The medium mixture (MEM) was used to mechanically resuspend the bone marrow by gentle aspiration through an 18-gauge needle and the suspension filtered through sterile nylon mesh gauze. The cells were then pelleted at 1000 rpm for 10 minutes, resuspended in MEM, and counted. In standard allogeneic reconstitution, RAMB was used for T-cell depletion of syngeneic B10 bone marrow (1:40 or appropriate dilution at  $10 \times 10^6$  cells/ml at 4°C. for 30 minutes). RAMB was prepared in the same manner as that described for immunization of rats with mouse brain in Section 6.1.2, supra, except that mouse brain was used to immunize rabbits. The allogeneic B10.BR bone marrow cells were either untreated, RAMB-depleted, anti-Thy1.2 depleted or hybridoma supernatant treated.  $10 \times 10^6$  donor bone marrow cells were pelleted and antibodies added 1:10 in 1 ml. The media were prewarmed to 37°C. so that the antibody incubation was performed at 37°C. for 30 minutes. Cells were then washed in MEM, spun at 1000 rpm for 10 minutes and resuspended in guinea pig complement at 37°C. for 30 minutes (Gibco Laboratories Life Technology, Inc., Grand Island, N.Y.). Cells were washed twice, counted and resuspended in MEM at the appropriate concentration to allow injection of 1 ml of total volume per animal. The RAMB-treated syngeneic cells were injected at  $5 \times 10^6$  /animal, whereas the allogeneic cells were given at  $15 \times 10^6$  /animal within 4-6 hours after irradiation of recipient animals at 9.5 Gy. Cell injections were via the lateral tail veins using a 27-gauge needle.